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File No.:	*Our Reference:*	*Owner/Applicant:*
PCT/EP2004/007530	AX02A15/P-WO	Axiogenesis AG

International application "Novel method for the preparation of embryoid bodies (EBs) and uses thereof" / Axiogenesis AG et al.

This is in response to the Written Opinion drawn up in accordance with Rule 43*bis*.1 PCT issued with the international search report on February 19, 2005, and to be considered to be a Written Opinion of the International Preliminary Examining Authority ("IPEA").

Herewith, a demand for international preliminary examination is made according to the enclosed form PCT/IPEA/401. The prescribed fees in the amount of € 1659.00 are to be debited from our deposit account No. 2800 0980; see also the enclosed Annex to form PCT/IPEA/401.

Furthermore, we enclose herewith an amended set of claims 1 to 42, which should form the basis for the international preliminary examination.

In the following, we would like to comment on the observations raised in the Written Opinion. In doing so, we will refer to the enclosed new set of claims.

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PA Patentanwalt · *Patent Attorney*
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ET European Trademark and Design Attorney

1. Amendments to the claims

1.1 Claims 1 to 30 correspond to original claims 1 to 30.

1.2 Amended claims 31 and 32 correspond to original claims 32 and 33 with the amendment that in accordance with original claim 31 they have been reformulated into method claims and made directly dependent on preceding claims 1 to 30.

1.3 Amended claim 33 corresponds to original claim 34 with the amendment that in accordance with original claim 31 the steps of the method for producing embryoid bodies have been incorporated into the claim by the corresponding reference to any one of claims 1 to 32.

1.4 Amended claims 34 to 39 correspond to original claims 35 to 40 with back references adjusted.

1.5 Amended claim 40 corresponds to original claim 42 with back references adjusted in view of the amendment to original claims 32 and 33; see section 1.2, supra. Furthermore, the subject matter of original claim 41 has been incorporated into the claim.

1.6 Amended claims 41 and 42 correspond to original claims 43 and 44 with back references adjusted.

It is respectfully submitted that the effected amendments do not introduce new matter but merely have been effected in order to direct the claimed subject matter to solution 1 as mentioned in item IV of the Written Opinion. This however does not mean that the objection for lack of unity raised by the Authority is justified. In particular, applicant reserves the right to reinstate any subject matter that may be no longer covered by the enclosed new set of claims and to pursue the original claims in national and regional stage applications derived from the present international application.

2. Unity (Rule 13.1 PCT)

In item IV of the Written Opinion, the present Authority referred to the objection for lack of unity raised in the International Search Report.

However, this objection does no longer apply to the enclosed amended set of claims;

see section 1, supra. In particular, all claims have been made directly dependent on claims 1 to 30, i.e. to the novel and inventive method for producing embryoid bodies based on agitation technique.

That the method of claims 1 to 30 and 34 to 40 can indeed be characterized as one continuous method is also supported by examples 3 and 4 of the present application, wherein the toxicity testing has been performed during further culturing of the embryoid bodies in accordance with the method as claimed in any one of claims 1 to 30.

Therefore, unity of invention should be acknowledged for the amended set of claims.

3. Clarity (Article 6 PCT)

In item VIII of the Written Opinion the Examiner argues that the wording "conditions allowing differentiation of the cells into at least one cell type" would allegedly refer to the underlying technical problem and therefore renders claim 17 not permissible under Article 6 PCT.

It is respectfully submitted that this observation is unjustified.

Article 6 PCT merely requires that the claims shall define the matter for which protection is sought, that they be clear and concise and that they shall be supported by the description.

These requirements are fully met by claim 17.

First, claim 17 is a dependent claim which simply comprises a further characterizing feature of the claimed culture method, i.e. that the culture conditions are such that the multi- or pluripotent cells differentiate into at least one cell type. This feature has a clear meaning to the person skilled in the art and there is no unambiguity to it.

Second, conditions allowing differentiation of multi- and pluripotent cells into a desired cell type are known per se; see for example the documents cited in the International Search Report and in the description of the present application, for example at page 17, line 21 to page 18, line 16.

For the above reasons, it is submitted that the contested claim meet the requirements of Article 6 PCT.

4. Sufficiency (Article 5 PCT)

In section 1 of item VII of the Written Opinion the Examiner argues that in view of the statement in document D1 at page 3, i.e. that conventional murine protocols for embryoid body (EB) formation failed with primate ES cells, only the formation of EBs from murine EBs would be enabled.

However, it is respectfully submitted that this mere statement in D1 is inappropriate to question sufficiency of the claimed method of the present invention.

As has been established for example in the EPO there must be serious doubts, preferably substantiated by verifiable facts that a claimed subject matter may be considered not to be sufficiently disclosed.

D1 at page 3 merely refers to those "conventional murine protocols" which by reference to the publications by Doetschman et al. and US patent 5, 914,268 date back until 1995 as the most recent publication cited in D1; see D1 at page 3, lines 8 to 11, with US patent 5,914,268 having a filing date of 1994.

However, since 1995 stem cell technology has considerably advanced. In this context, reference can be made to document D2, filed in 2002, i.e. seven years later than the publication by Doetschman et al., which provides full citations for corresponding references which are more recent and therefore more appropriately define the state of the art than the mere statement in D1; see also tables 10 and 11 in D2 at page 17, which provide examples of cell types inter alia derived from human embryonic stem cells. Thus, even if the statement in D1 was true in 1995 or at the priority date of D1, i.e. at the beginning of 2000, it does certainly not reflect the state of the art at the priority date of the present application, i.e. mid 2003.

Furthermore, it should be acknowledged that the method of the present invention is aiming at improving the production of embryoid bodies by the process features as recited in claim 1 and its dependent claims, rather than claiming that embryoid bodies can now be easily produced from any multi- and pluripotent cell from any species.

As such, the method of the present invention is generally enabled.

Moreover, even if in some cases the generation of EBs from ES cells of a given species may fail, according to establish case law, in particular in the EPO, the fact that the scope of a claim may encompass embodiments which have not yet been enabled, does not invalidate the claim. Only if essential features are missing in the claim necessary to put the claimed invention into practice, sufficiency may be denied.

However, as explained above, and demonstrated in the examples this is certainly not the case for the claimed method of the present invention.

Given the fact that the methods of the present invention can indeed be generally applied to stem cell derived embryoid bodies and in the absence of evidence to the contrary, the applicant should be given the benefit of doubt.

For the above reasons, it is requested that the objections under Article 5 PCT be withdrawn.

5. Novelty (Article 33(2) PCT)

In section 1 of item V of the Written Opinion, the Examiner argues that claims 1 to 7 lack novelty over document D1 [*sic*], probably meaning D2, i.e. US2003/119107 A1 which according to the Examiner discloses the importance of controlling cell aggregation during formation of embryoid bodies from ES cells and agitation of the culture system.

While the individual statements may be true, document D2 nevertheless does not disclose the method for producing embryoid bodies as characterized in claim 1 of the present application.

Claim 1 of the present application relates to a method for producing embryoid bodies (EBs) from multi- or pluripotent cells comprising

(a) agitation of a liquid suspension culture of multi- or pluripotent cells in a container until generation of cell aggregates; and

(b) optionally diluting the suspension, and further agitation of the suspension until formation of EBs.

As discussed in the description, for example, at page 10, line 14ff and demonstrated in the examples the method of the present invention is based on the surprising finding that ES cells when agitated during culture rather than for example being stirred, said ES

cells preferentially form cell aggregates which upon further culturing, with an optional dilution step, mature into the formation of embryoid bodies. This novel finding has find its expression in features (a) and (b) of claim 1.

In contrast, the essence of the teaching of D2 is based on experiments demonstrating the generation of spheroids, wherein the spheroid forming cells are encapsulated and cultivated in a stirred liquid culture; see example 2b. Through this measure, aggregation of the primary ES cells - in contrast to the present invention - is prevented; see for example the illustration in figure 2 of D2 and the description, for example at paragraph [0063] and subsequent paragraphs as well as the examples which continuously teach to encapsulate the ES cells and to keep them in stirred culture or suspension bioreactors; see for example paragraph [0113] and [0161], respectively. Hence, throughout all of the examples of D2 including the tables and figures reference is made to encapsulated stirred cultures.

Thus, while the method of the present invention as claimed in claim 1 facilitates aggregation of ES cells, D2 just teaches the opposite, i.e. preventing aggregation of the primary ES cells via encapsulation.

From the overall disclosure and the examples it is thus clear that the terms "controlling cell aggregation" in paragraph [0054] and "agitation of the system" in paragraph [0053] have merely been mentioned in order to broaden the scope of the claims.

However, as is well established for example in the EPO, there is a difference between the scope of the claims of a patent application or a patent and the actual disclosure the person skilled in the art can derive from the description and the examples of the patent document. In this regard, it is undisputable that the actual teaching the person skilled in the art can derive from D2 is to prevent the ES cell aggregation in the culture by encapsulation and to use stirring cultures.

For the above reasons, the subject matter of claim 1 is novel over D2.

The same mutatis mutandis applies to claims 2 to 42, which are directly or indirectly dependent of claim 1.

6. Inventive Step (Article 33(3) PCT))

In section 2 of item V of the Written Opinion claims 8 to 13 and 42 to 44 are objected for alleged lack of inventive step.

However, it is submitted that this objection is based on the Examiner's incorrect assessment of document D2 in relation to claims 1 to 7 as has been explained in section 4, supra.

5.1 The technical problem underlying the present invention

As stated in the present application, the problem underlying the present invention is to provide reliably, easy and cost-effective methods for producing embryoid bodies in sufficient quality and quantity. The solution to this technical problem is achieved by providing the embodiments characterized in claim 1, and its dependent claims.

Thus, the present application teaches to agitate a liquid suspension culture of multi- or pluripotent cells, for example ES cells in a container in order to facilitate the formation of aggregates by said cells, which after an optional dilution step and further agitation of the suspension results in homogenous cell aggregation formation and a high yield of embryoid bodies.

The examples of the present application demonstrate that the problem has been solved in accordance with the claimed method.

5.2 The closest prior art

The closest prior art may bee seen in document D2 which seems to relate to a similar but not identical problem of generating large numbers of embryonic stem cell derived tissue; see D2 for example at the abstract.

According to D2, this problem may be overcome by encapsulating individual ES cells and thus preventing them from aggregation which in accordance with the teaching of D2 should result in the formation of embryoid bodies from each individual encapsulated ES cells; see for example paragraph [0054] and particularly the examples and figure 2 of D2.

Thus, D2 just teaches the opposite from the present invention, i.e. preventing the formation of aggregates versus facilitating the same. Accordingly, D2 neither teaches

nor suggests the method of the present invention.

5.3 The method of the present invention provides advantages over the method of D2

As has already been discussed in section 4, supra, D2 teaches to take certain measures in order to prevent ES cells from aggregation, exemplified by encapsulation while in the description further measures are described; see paragraphs [0063] to [0069]. All these methods for preventing cell aggregation require considerable efforts and are cumbersome. Furthermore, the required additional step of for example encapsulation provides a further source of error in the method of the production of embryoid bodies.

In contrast, the method of the present invention is simple to perform and does not require any particular manipulation of the ES cells and/or change in culture medium.

Instead, the method of the present invention can just be conveniently performed in containers such as flasks and the resultant embryoid bodies can be instantly used for, e.g., toxicity tests; see examples 3 and 4 of the present application.

Accordingly, the method of the present invention can be more easily performed as well as in a more cost-effective manner compared to the method taught in D2.

For the above reasons, inventive step for all claims should be acknowledged.

7. Requests

With the above explanations and the amendments to the claims, it is submitted that the applicant has met the requirements of the PCT. It is therefore requested that the Authority's objections be withdrawn and that a favourable IPER be issued.

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Amended claims 1 to 42
Form PCT/IPEA/401
Annex to Form PCT/IPEA/401